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(71) Sökande Gyros AB, Uppsala SE  
Applicant (s)

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*Marita Öun*  
Marita Öun

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## A COLLECTION OF MICRO SCALE DEVICES

### TECHNICAL FIELD

The present invention relates to a collection of one or more microfluidic devices, each of  
5 which carries one or more microchannel structures in which there is a reaction microcavity  
containing a solid phase with an immobilized affinity ligand.

Each microchannel structure is primarily intended for performing an affinity protocol  
utilizing an affinity reaction comprising a solute S and its affinity counterpart  $AC_S$  that  
10 typically is immobilized or immobilizable to the solid phase. The reaction may comprise  
formation or dissociation of an affinity complex  $AC_S-S$ . The affinity complex may  
comprise other affinity reactants in addition to S and  $AC_S-S$ .

The affinity protocol may be an affinity assay such as an enzyme assay, an immune assay,  
15 a cell based assay, a hybridization assay and other affinity assays in which an  
uncharacterized analyte or some other reaction variable is to be characterized. The affinity  
reaction may also be part of a protocol for which the main objectives are separation,  
purification and/or enrichment.

20 The term "solute" includes true solutes, microorganisms such as viruses, suspended cells,  
suspended cell parts and various other reactants that are in dissolved or colloidal form and  
sufficiently small to be transported by liquid flow through the bed. The solute S may be the  
analyte of an original sample or an analyte-derived entity formed before the affinity  
reaction between the solute S and its affinity counterpart  $AC_S$ .

25

The term "microfluidic device" means that a liquid flow can be used for transporting  
various kinds of reactants, samples, buffers etc in the microchannel structures of the  
device.

30 The terms "micro" in "microchannel", "microconduit", "microvolumes" etc., contemplate  
that a channel structure comprises one or more cavities and/or conduits that have a cross-  
sectional dimension that is  $\leq 10^3 \mu m$ , preferably  $\leq 5 \times 10^2 \mu m$ , such as  $\leq 10^2 \mu m$ . The

volume of a liquid aliquot to be processed within the device is typically in the nanolitre (nl) range (which includes the picolitre (pl) range). The nl-range has an upper end of 5,000 nl but relates in most cases to volumes  $\leq 1,000$  nl, such as  $\leq 500$  nl or  $\leq 100$  nl.

- 5 Patent publications cited herein are incorporated by reference in their entirety.

### BACKGROUND PUBLICATIONS

- WO 0275312 (Gyros AB) focuses on affinity assays for the characterization of reaction variables by binding an affinity reactant to a solid phase that comprises the counterpart to  
10 the affinity reactant or by releasing an affinity reactant from a solid phase that comprises an immobilized affinity complex between the affinity reactant and its affinity counterpart. Various immobilization techniques are suggested, e.g. covalent binding, physical adsorption, bioaffinity binding including streptavidin-biotin, etc.

- 15 US 5,726,026 (Univ. Pennsylvania) and US 5,928,880 (Univ. Pennsylvania) describe a microfluidic device that may comprise a solid phase in the form of particles to which streptavidin have been pre-bound. The particles can be sensitized with biotinylated antibody.

### 20 BACKGROUND PROBLEMS

- It would be of big economic benefit if the same or similar standard procedures could be used for the manufacture of devices intended for a range of assay and/or separation protocols, a number of concentration ranges, a range of analytes etc. This includes that it is important to provide microfluidic devices that enable the measurement of analytes of  
25 low activity and/or concentration in small volume samples, e.g. concentrations in the nanomolar range including the picomolar range in  $\mu$ l- including nl-samples. In this context the nanomolar range comprises  $\leq 1,000 \times 10^{-9}$  M including the picomolar range  $\leq 1,000 \times 10^{-12}$  M.  $\mu$ l-samples comprises  $\leq 1,000$   $\mu$ l including nl-samples  $\leq 5,000$  nl.
- 30 It is often both difficult and critical to find affinity reagents/reactants, such as antibodies, of a quality that will give a sufficiently high analytical sensitivity and specificity, diagnostic sensitivity and specificity etc required by diagnostic and medical needs. The

affinity and the specificity of one reactant, for instance, may be difficult to match with other reactants of a protocol in order to comply with preset specifications. It would thus be beneficial to provide assay systems and methodologies that inherently increase features such as sensitivity to thereby make more affinity reagents available without compromising earlier decided specifications.

### OBJECTS OF THE INVENTION

A first main object is to provide improved microfluidic devices that solve the problems discussed above. This includes providing methods for manufacturing and use of the devices.

A second main object is to provide a product offer that is intended for performing affinity reactions of a range of different affinity reactants, such as analytes, which may be present in the same or different concentration intervals by a range of different assay protocols. The offer can be envisaged as a number of microchannel structures which are portioned into sets where each set is dedicated to a particular combination of analytes, and/or a particular range of concentrations of the same or different analytes, and/or particular combinations of different affinity protocols.

A third main object is to provide a system for affinity assays that will expand the range of affinity reactants/reagents that will fulfill performance requirements on analytical and diagnostic sensitivity and specificity of an affinity assay.

In this context the term "different affinity protocols" refers to a number of affinity protocols each of which differ with respect to at least one member selected amongst (a) the reactants used, (b) the sequence of steps, and (c) incubation times, for instance contact time with a solid phase, from any of the other protocols.

### THE INVENTION

We have discovered that affinity immobilized capture reactants may be more efficient than covalently immobilized capture reactants for capturing solutes from a liquid phase. The capture reactant and the solute are affinity counterparts. If the capture, for instance, is

carried out under flow conditions, in particular combined with non-diffusion limiting conditions, in the reaction microcavity of a microchannel structure as described in WO 0275312 (Gyros AB) with an excess of solid phase bound capture reactant, the solute will appear on the solid phase as a more concentrated band when the capture reactant is affinity immobilized compared to immobilized covalently. An increased analytical sensitivity is suggested if the capturing is part of an affinity assay.

Indications of increased analytical sensitivity for affinity immobilization have also been deduced from dose-response graphs.

10

We have realized that our discovery may have an impact on the selection of immobilization technique if one would like to provide a product offer as defined for the second main object of the invention.

15 We have further elaborated this discovery and recognized that assaying of analytes of high as well as of low concentrations can be accomplished with high accuracy by using the proper combination of:

- a) Flow conditions during the capturing step, i.e. during the formation of the immobilized complex  $AC_S-S$ .
- 20 b) A generic immobilizing binding principle based on a common affinity ligand (L e.g. streptavidin) immobilized to the solid phase and/or one or more conjugates represented by a common binder directed towards the common ligand L (immobilizing binder B, e.g. biotin) that for each conjugate is attached to an affinity counterpart  $AC_S$  of a particular solute S that varies for the different conjugates.
- 25 c) Proper selection of the binding capacity of the solid phase for the conjugate (or for the immobilizing binder in unconjugated form) and saturation of the solid phase with a conjugate that is capable of capturing a particular solute S.
- d) Balancing the flow conditions (flow rate and/or residence time) in relation to the affinity pair ( $AC_S$  and S) to achieve non-diffusion limiting conditions for optimal
- 30 enrichment of the solute on the solid phase.

The capacity/reaction microcavity and/or the capacity/volume unit of the solid phase in a reaction microcavity for binding the conjugates (e.g. biotin conjugates) then determine the kind of solute and analyte, the concentration range of solute/analyte, and/or the assay protocols etc, a particular solid phase/microchannel structure may be used for.

5

#### **FIRST ASPECT: COLLECTION OF MICROFLUIDIC DEVICES**

The main aspect of the invention thus is a collection of one or more microfluidic devices which together carry a plurality of microchannel structures each of which comprises a reaction microcavity in which there is a solid phase with an immobilized affinity ligand L.

10 The characteristic feature of the set is that

- i) the plurality of microchannel structures is divided into two or more sets of microchannel structures, and
- ii) the affinity ligand L is directed to the same counterpart (binder, B) independent of subset, and

15 iii) the sets differ from each other in that

- a) the capacity/reaction microcavity, and/or the capacity/unit volume of the solid phase in a reaction microcavity for binder B, and/or
  - b) the base matrix of the solid phase material
- differ between the sets but is equal within each set, and/or the base matrix .

20

The collection is adapted for separately performing one or more affinity protocols that differ with respect to the reactants involved and/or the order of addition of the reactants and/or the concentration range in which the reactants are used. Each of the different protocols utilizes an affinity reaction between

25 a) a solute S, and

b) a conjugate comprising (i) binder B, and (ii) an affinity counterpart AC<sub>S</sub> to the solute S.

#### **Collection of microfluidic devices versus sets of microchannel structures.**

At least one of the microfluidic devices of the innovative collection comprises

- 30 a) at least two different sets of microchannel structures, and/or
- b) only one set of microchannel structures.

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Alternative (b) does only apply if there are two or more of the microfluidic devices in the collection.

One, two or more of the sets of the microchannel structures on a microfluidic device of the innovative collection comprise at least two microchannel structures. In a typical case this apply to all the sets that are represented on a particular device.

The collection may also contain microfluidic devices that are multiplicates, i.e. several devices that have the same combination of sets. Multiplicates of a particular device may be 10 copies or may differ with respect to other features, e.g. (1) total number of microchannel structures, and/or (2) the number of microchannel structures of a particular set, (3) grouping and/or positioning of groups/microchannel structures on a device (see below), (4) combination of functional units, (5) number of microchannel structures that do not affinity ligand L, (6) etc.

15

A device of the innovative collection may contain microchannel structures of one, two, three or more of the different sets. On a microfluidic device that comprises several sets, microchannel structures of the same set are preferably placed together in one or more groups with each group being located to a particular area/subarea of the device. The 20 structures within the same group and/or set are preferably fluidly equivalent. On a centrifugally based microfluidic device, for instance, the microchannel structures of a set or a group may be located in a defined sector or in an annular zone. The corresponding parts of the microchannel structures of a group are then preferably at the same radial distance from the spin axis of the device.

25

The term "fluidly equivalent microchannel structures" means that the structures have the same combination of fluid functions and/or can be processed in parallel by a commonly applied force for driving a liquid flow through them.

30 In preferred variants each of one or more, preferably all, of the reaction microcavity(ies) which contains the affinity ligand L and is part of the same set communicates in preferred variants in the upstream direction with a volume-metering unit. This applies to one or

- more, preferably all, of the sets. Each volume-metering unit is typically part of an inlet arrangement for liquid that comprises an inlet port for receiving liquid and/or particles that are dispensed to the device. The volume-metering units for two or more microchannel structures of different sets, or more preferably of the same set or group, may define a
- 5 common distribution manifold which in turn may or may not be part of a common inlet arrangement with one or more common inlet ports. One, two or more, preferably all of the microchannel structures of a group defined by a common inlet arrangement may also have a separate inlet arrangement with one, two or more inlet ports per microchannel structure. Each group of microchannel structures defined by a common inlet arrangement and/or
- 10 distribution manifold is preferably located to a particular subarea of the device. Useful inlet arrangements with volume-metering units, distribution manifolds and/or inlet ports have been presented in WO 0274438 (Gyros AB), WO 0275312 (Gyros AB), WO 0275775 (Gyros AB) and WO 0275776 (Gyros AB).
- 15 Grouping of the microchannel structures of a device into groups may also be according to criteria other than sets and/or common inlet functions. Typical other groupings are according to other common functions, such as common waste functions, common venting functions, type of mixing function, type of heating function etc. See below.
- 20 The number of sets of microchannel structures of the innovative collection is typically 2, 3, 4, 5, 6, 7 ..... 10 or more.

- In addition to the difference in binding capacity and/or base matrix, the sets may also have other differences, for instance with respect to other functional units and how they are
- 25 connected.

#### **Immobilizing affinity pair (L and B)**

- The affinity ligand L and its counter part binder B are called an immobilizing affinity pair. Typically the immobilizing binding pair should be selected such that, except for the desired
- 30 affinity binding to each other, they should be essentially devoid of other binding abilities during the conditions used, for instance towards other reactants.



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Preferred immobilizing affinity pairs (L and B) typically have affinity constants ( $K_{L-B} = [L][B]/[L-B]$ ) that are at most equal to or  $\leq 10$  times or  $10^2$  times or  $\leq 10^3$  times larger than the corresponding affinity constant for streptavidin and biotin. This typically will mean affinity constants that roughly are  $\leq 10^{-13}$  mole/l,  $\leq 10^{-12}$  mole/l,  $\leq 10^{-11}$  mole/l and  $\leq 10^{-10}$  mole/l, respectively.

It is believed that it is advantageous that the immobilized ligand L has two or more binding sites for the immobilizing binder B, and/or the immobilizing binder B has one, two or more binding sites for the ligand L (or vice versa).

10

The immobilizing affinity pair may in principle be selected amongst any affinity pairs for which the two affinity counterparts have a sufficiently strong affinity to each other and are essentially inert towards reaction with other reactants during the conditions used for a particular application. Particular examples of immobilizing affinity pairs are a)

15 streptavidin/avidin/ neutravidin and a biotinylated reactant (or vice versa), b) antibody and a haptenylated reactant (or vice versa), c) an IMAC group and an oligopeptide containing a sequence of histidyl and/or histidyl residues (i.e. an IMAC motif) linked to a reactant, etc.

The preference at the priority date is to select L and B amongst biotin-binding compounds and streptavidin-binding compounds, respectively, or vice versa. Streptavidin and neutravidin are believed to work best as the immobilized affinity ligand L.

The affinity ligand L should be attached more firmly to the solid phase than the binder B is affinity bound to the affinity ligand L. This is valid for the conditions applied during  
25 binding of the solute S to the solid phase via the immobilizing binding pair, i.e. via the immobilizing conjugate B-ACs. This typically means covalent immobilization of the affinity ligand L although also adsorptive bonds involving electrostatic attraction, van-der Waals bonding etc may be used. Utilizing polymeric carriers carrying both the affinity ligand and a plurality of groups that are capable of interacting with a counter structure on  
30 the surface of the solid phase may accomplish strong attachment of the affinity ligand L via adsorptive forces, for instance.

Binding capacity can be measured as the amount affinity ligand L in mole per unit volume, disregard blocking and destruction of binding sites caused by the immobilization. With this measure suitable binding capacities will typically be found within the interval of 0.001 - 3000 pmole, such as 0.01 - 300 pmole, per nl solid phase in bed form saturated with liquid.

5 For instance, if 0.1 pmole streptavidin per nl has been immobilized this corresponds 0.4 pmole/nl biotin-binding sites. The conversion factor four is because has four binding sites for biotin per molecule.

Binding capacity can also be measured as actual binding capacity, i.e. mole active binding

10 sites per unit volume of the solid phase containing the immobilized affinity ligand in bed form saturated with liquid. This kind of binding capacity will depend on the immobilization technique, the pore sizes of the solid phase, the size of the entity to be immobilized, the material and design of the solid phase etc. Ideally the same ranges apply for the actual binding capacity as for the total amount of binding sites (as defined above).

15

Measurements of actual binding capacities can be carried out according to principles well known in the field. This typically means that affinity ligands of the solid phase is saturated with an excess binder molecules whereafter the amount bound is measured, for instance directly on the solid phase or after elution. To facilitate measurement labeled forms of

20 binders may be used for instance by the use of mixtures of labeled and unlabelled binder.

The actual binding capacity of a solid phase will depend on the difference between the ability of an affinity ligand and the corresponding binder B to penetrate the solid phase. For porous particles, a larger binder may for instance show a decreased actual binding

25 capacity compared to a smaller binder if the affinity ligand and its concentration on the solid phase is kept the same. The above-discussed actual binding capacities therefore primarily refer to binding/capturing of the binder B in its base form, e.g. unconjugated and/or underivatized.

30 In the case the solid phase is an inner wall of the reaction microcavity, the volume of the solid phase is taken as the volume of the reaction microcavity.

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Huvudföreläsningen

The optimal range of binding capacities for a particular kind of experiments depends on a number of factors, e.g. kind of solute and/or the original analyte and/or the concentration range in which they are measured, amount of liquid containing the solute/analyte, immobilizing affinity pair, kind of solid phase, e.g. porosity and its base material, size of conjugate (B-ACs), ratio between number of binding structures for L and number of binding structures for S (of the conjugate), etc. Testing by trial and error is at the moment the safest way to optimize the binding capacity in relation to a particular experiment.

Binding capacities can also be measured per bed volume. The intervals within which suitable binding capacities of this kind can be found are deducible from the capacity ranges given above combined with the intervals for the bed volumes given below.

The differences between the sets in binding capacity per volume unit and reaction microcavity is typically a factor  $\geq 1.2$  for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 etc sets, such as for 5-95%, such as for  $\geq 10\%$  or  $\geq 25\%$  or  $\geq 40\%$  or  $\geq 55\%$  or  $\geq 70\%$  or  $\geq 85\%$  and/or for  $\leq 85\%$  or  $\leq 70\%$  or  $\leq 55\%$  or  $\leq 40\%$  or  $\leq 25\%$  or  $\leq 19\%$ , of the sets of the collection compared to the binding capacity per volume unit for the set having the lowest binding capacity. The factor may also be  $\geq 2$  or  $\geq 4$ , such as  $\geq 10$  or  $\geq 50$  or  $\geq 90$  or  $\geq 500$  for the same number and the percentages of the sets as when the factor is  $\geq 1.2$  with due account taken so that percentages below or above a factor always add to 100 %.

The same intervals for the various factors and the percentages apply also for the binding capacity per reaction microcavity/bed.

## 25 The affinity counterpart AC<sub>s</sub> and the solute S

The affinity counterpart AC<sub>s</sub> is capable of binding to the solute S by affinity. This kind of binding is typically based on: (a) electrostatic interactions, (b) hydrophobic interactions, (c) electron-donor acceptor interactions, and/or (d) bioaffinity binding.

30 Bioaffinity binding is a subgroup of affinity binding that typically comprises a combination of interactions.

The affinity counterpart AC<sub>s</sub> may thus:

- (a) be electrically charged or chargeable, i.e. contains positively charged nitrogen (e.g. primary, secondary, tertiary or quaternary ammonium groups, and amidinium groups) and/or negatively charged groups (e.g. carboxylate groups, phosphate groups, phosphonate groups, sulphate groups and sulphonate groups); and/or
- (b) comprise one or more hydrocarbyl groups and other hydrophobic groups; and/or
- (c) comprise one or more heteroatoms (O,S,N), possibly linked to hydrogen and/or an sp<sup>2</sup>- and/or sp<sup>3</sup>-hybridised carbon, and/or
- (d) comprise a combination of features (a)-(c).

10

The affinity counterpart AC<sub>s</sub> may, depending on the features of the solute, be selected amongst reactants that are members of a bioaffinity pair. Typical bioaffinity pairs are a) antigen/hapten and an antibody, b) complementary nucleic acids, c) immunoglobulin-binding protein and immunoglobulin (for instance IgG or an Fc-part thereof and protein A or G), d) lectin and the corresponding carbohydrate, e) biotin and (strept)avidin, e) members of an enzymatic system (enzyme-substrate, enzyme-cofactor, enzyme-inhibitor etc), f) a sequence of histidyl, cysteinyl, phosphate etc and an IMAC group (immobilized metal chelate), etc. Antibody includes antigen binding fragments and mimetics of antibodies and their fragments and recombinant constructs. The term "bioaffinity pair" includes also affinity pairs in which one or both of the members are synthetic, for instance mimicking one or both of the members of a native bioaffinity pair.

The term "affinity reactant" also includes a reactant that is capable of reversible covalent binding, for instance by disulfide formation. This kind of reactants typically exhibits a HS- or a -S-SO<sub>n</sub>- group (n = 0,1 or 2, free valences bind to carbon). See US 5,887,997 (Batista), US 4,175,073 (Axén et al), and 4,563,304 (Axén et al).

The affinity counterpart AC<sub>s</sub> may also be a catalytic system or a member of a catalytic system, such as a catalyst, a cocatalyst, a cofactor, a substrate or cosubstrate, an inhibitor, a promotor etc. For enzymatic systems the corresponding members are enzyme, cocatalyst, cofactor, coenzyme, substrate, cosubstrate etc. The term "catalytic system" also includes linked catalytic systems, for instance a series of systems in which the product of the first

system is the substrate of the second catalytic system etc and whole biological cells or part of such cells.

The affinity counterpart  $AC_s$  should be selected to have the appropriate selectivity and specificity for binding the solute to the solid phase material in relation to an intended application. General methods and criteria for the proper selection of affinity reactants and reaction conditions are well known in the field. This also applies to the selection of affinity ligand  $L$  and the binder  $B$ .

- 10 The affinity constant ( $K_{S-AC} = [S][AC_s]/[S-AC_s]$ ) for the formation of the complex comprising the affinity counterpart  $AC_s$  and the solute  $S$  is an important criterion for optimizing an application. The demand on this affinity constant varies depending on application. For affinity assays the affinity constant is typically  $\leq 10^{-8}$  mole/l or  $\leq 10^{-9}$  mole/l. This kind of assays typically includes that the solute is reacted with immobilized
- 15  $AC_s$  under flow conditions and related to the amount of an analyte in an animal or biological sample (animal or biological sample include samples from mammals, such as human and other animal patients, and from experimental animals). This does not exclude that affinity counterparts having weaker affinities may be used for this kind of samples, other samples and affinity assays, and other applications. Thus depending on application
- 20 the affinity constant may be relatively large, such as up to  $10^{-3}$  mole/l or up to  $10^{-4}$  mole/l or up to  $10^{-5}$  or up to  $10^{-7}$  mole/l.

The affinity constants refer to values obtained by a biosensor (surface plasmon resonance) from Biacore (Uppsala, Sweden), i.e. with the affinity reactant ( $AC_s$  and  $L$ ) immobilized to

25 a dextran-coated gold surface.

- At least one of the members of an affinity pair, in particular a bioaffinity pair, typically exhibits a structure selected amongst: a) amino acid structure including peptide structure such as poly and oligopeptide structure, b) carbohydrate structure, c) nucleotide structure
- 30 including nucleic acid structure, d) lipid structure such as steroid structure, triglyceride structure etc. This kind of structures may be present in  $L$ ,  $B$ ,  $AC_s$  and  $S$  as well as in other affinity reactants used.

### **The conjugate**

The term "conjugate" primarily refers to covalent conjugates, such as chemical conjugates and recombinantly produced conjugates (where both the moieties and also a possible linker  
5 have peptide structure).

The term in "unconjugated form" with respect to the common binder B means a form that does not exhibit any binding site for the solute of interest. The term includes also the reagents necessary for a conjugation with AC<sub>s</sub>, and that B is in a form suitable for  
10 conjugation with AC<sub>s</sub>. This typically means that B in unconjugated form in the context of the invention exhibits a functional group that is electrophilic or nucleophilic and is capable of forming covalent links with functional groups of an affinity reactant such as AC<sub>s</sub>.  
Electrophilic, nucleophilic and functional groups can be selected, for instance, amongst amino groups and other groups comprising substituted or unsubstituted -NH<sub>2</sub>, carboxy  
15 groups (-COOH/-COO<sup>-</sup>), hydroxy groups, thiol groups, keto groups etc. The structures discussed above for potentially being present in affinity reactants can also be utilized for conjugation if being present in either one or both of B and AC<sub>s</sub>.

The conjugate may be present in the collection either predisposed to the microchannel  
20 structures of a device (i.e. to the interior of a device), or kept as a separate entity of the collection outside the devices. If predisposed, the conjugates may be present in the reaction microvacities and affinity bound to the solid phase via the immobilized affinity ligand L, or in some other location within the microchannel structures/devices. If present outside the devices the conjugate is typically in a separate package as a solution or in dry  
25 form, e.g. lyophilized or dried at atmospheric or reduced pressure. In still another alternative the conjugate is provided from other sources, for instance synthesized by the customer.

The binder (B) in unconjugated form is typically separate from the devices, although one  
30 can envisage preloading it to the microchannel structures (i.e. to the interior of the devices) for preparing the conjugates within the devices at the time of use. Additional reagents that

are necessary for preparing the conjugates, for instance activation reagents, are typically also separate from the devices in the collection.

## 5 Reaction microcavity and solid phase

The reaction microcavity is defined as the part of a microchannel structure in which the solid phase is present. This means that for solid phases in the form of porous beds, the bed volume and the reaction microcavity will coincide and have the same volume. If the solid phase is the inner wall of a microconduit, the reaction microcavity is defined as the volume  
10 between the most upstream and the most downstream end of the solid phase.

The reaction microcavity has at least one cross-sectional dimension that is  $\leq 1,000 \mu\text{m}$ , such as  $\leq 500 \mu\text{m}$  or  $\leq 200 \mu\text{m}$  (depth and/or width). The smallest cross-sectional dimension is typically  $\geq 5 \mu\text{m}$  such as  $\geq 25 \mu\text{m}$  or  $\geq 50 \mu\text{m}$ . The volume of the reaction  
15 microcavity is typically in the nl-range, such as  $\leq 5,000 \text{ nl}$ , such as  $1,000 \text{ nl}$  or  $\leq 500 \text{ nl}$   $\leq 100 \text{ nl}$  or  $\leq 50 \text{ nl}$  or  $\leq 25 \text{ nl}$ .

The geometric form for a reaction microcavity is typically essentially the same for all of the reaction microcavities that define the sets of the innovative collection, in particular for  
20 microcavities belonging to the same set. Sets that differ with respect to binding capacity per microcavity but have equal binding capacity per unit volume may be obtained if the volume of each reaction microcavity of each set is different from the corresponding volume of any of the other sets.

25 The solid phase is ideally the same for all sets but may vary between and/or within the sets. One can envisage that in order to run experiments for certain selected solutes (analytes), concentration ranges, samples, protocols etc, optimal conditions will require selected solid phases that do not fit to other solutes, other concentration ranges, samples, protocols etc. Differences may relate to the base matrix, such as  $K_{av}$ -values, and/or compositions of  
30 solid phase, and/or presence or absence of pores permitting convective and/or diffusive mass transport of reactants, etc. Differences may also relate immobilization techniques and the like.

The solid phase in a reaction microcavity may be a porous bed or one or more inner walls of the reaction microcavity. Examples of porous beds are a) a population of porous or non-porous particles that are packed to a bed, or b) a porous monolith.

5

The term "porous particles" have the same meaning as in WO 0275312 (Gyros AB).

Suitable particles may be spherical or spheroid (beaded) or non-spherical. Suitable mean diameters for particles used as solid phases are typically found in the interval of 1-100  $\mu\text{m}$  with preference for mean diameters that are  $\geq 5 \mu\text{m}$ , such as  $\geq 10 \mu\text{m}$  or  $\geq 15 \mu\text{m}$  and/or  $\leq 50 \mu\text{m}$ . Also smaller particles can be used, for instance with mean diameters down to 0.1  $\mu\text{m}$ . The outlet of the reaction microcavity and the particles should match each other so that the particles can be retained in the reaction microcavity. See for instance WO 0275312 (Gyros AB). Certain kinds of particles, in particular particles of colloidal dimension, may agglomerate. In these cases the size of the agglomerate should be in the intervals given even if the agglomerating particles as such are below. Diameters refer to the "hydrodynamic" diameters.

Particles to be used may be monodisperse (monosized) or polydisperse (polysized) in the same meaning as in WO 0275312 (Gyros AB).

The solid phase material may or may not be transparent.

The base material of a solid phase may be made of inorganic and/or organic material.

Typical inorganic materials comprise glass and typical organic materials comprise organic polymers. Polymeric materials comprise inorganic polymers, such as glass, and organic polymers that may be of synthetic or biological origin (biopolymers). The term biopolymer includes semi-synthetic polymers in which there is a polymer backbone derived from a native biopolymer. Typical synthetic organic polymers are cross-linked and are often obtained by the polymerisation of monomers comprising polymerisable carbon-carbon double bonds. Examples of suitable monomers are hydroxy alkyl acrylates and corresponding methacrylates, acryl amides and methacrylamides, vinyl and styryl ethers,



alkene substituted polyhydroxy polymers, styrene, etc. Typical biopolymers may or may not be cross-linked. In most cases they exhibit a carbohydrate structure, e.g. agarose, dextran, starch etc.

- 5 The term "hydrophilic" for porous beds means that the solid phase is capable of absorbing water. If the solid phase is the inner wall of a reaction microcavity "hydrophilic" primarily means that the water contact angle is within the limits specified for hydrophilicity (wettability) elsewhere in this specification. Surfaces that are to be in contact with aqueous liquids shall also expose a plurality of polar functional groups which each has a heteroatom
- 10 selected amongst oxygen and nitrogen, for instance. Appropriate functional groups can be selected amongst hydroxy groups, ethylene oxide groups  $(-X-[-CH_2CH_2O-])_n$  where  $n$  is an integer  $> 1$  and  $X$  is nitrogen or oxygen), amino groups, amide groups, ester groups, carboxy groups, sulphone groups etc, with preference for those groups that are essentially uncharged independent of pH, for instance within the interval of 2-12.

15

- If the base material of a solid phase material is hydrophobic or not sufficiently hydrophilic, e.g. is based on a styrene (co)polymer, the surfaces that are to be in contact with an aqueous liquid may be hydrophilized. Typical protocols comprise coating with a compound or mixture of compounds exhibiting polar functional groups of the same type as
- 20 discussed above, treatment by an oxygen plasma etc.

#### General features of the microfluidic device

The number of microchannel structures containing the solid phase with affinity ligand  $L$  on the same microfluidic device is typically  $\geq 10$ , e.g.  $\geq 25$  or  $\geq 90$  or  $\geq 180$  or  $\geq 270$  or  $\geq 360$ .

- 25 As discussed above the microchannel structures of a device may be divided in subgroups.

The number of microchannel structures in each subgroup is typically in the interval 1-99 %, such as 5-50 % or 5-25 % or 10-50%, of the total number of microchannel structures of the device. This typically means that each subgroup comprises from 3-15 or 3-25 or 3-50 microchannel structures.

30

A microchannel structure may comprise one, two, three or more functional parts selected among: a) inlet arrangement comprising for instance an inlet port/inlet opening, possibly

together with a volume-metering unit, b) microconduits for liquid transport, c) reaction microcavity; d) mixing microcavity; e) unit for separating particulate matters from liquids (may be present in the inlet arrangement), f) unit for separating dissolved or suspended components in the sample from each other, for instance by capillary electrophoresis, chromatography and the like; g) detection microcavity; h) waste conduit/microcavity; i) valve; j) vent to ambient atmosphere; etc. A functional part may have more than functionality, e.g. reaction microcavity and a detection microcavity may coincide. Various kinds of functional units in microfluidic devices have been described by Gyros AB/Amersham Pharmacia Biotech AB: WO 9955827, WO 9958245, WO 02074438, WO 0275312, PCT/SE02/01539, PCT/SE02/01701 and by Tecan/Gamera Biosciences: WO 0187487, WO 0187486, WO 0079285, WO 0078455, WO 0069560, WO 9807019, WO 9853311.

As already discussed for common inlet arrangements, the innovative microfluidic device may also comprise other common microchannels/micro conduits/microfluidic functions connecting two or more microchannel structures. Common channels including their various parts such as inlet ports, outlet ports, vents, etc., are considered part of each of the microchannel structures they are communicating with. In a similar manner as for the common inlet arrangements, these other common functions may be the basis for dividing the microchannel structures of a device into groups and/or designating different groups of the microchannel structures of a device to different subareas of a microfluidic device. These kind of groups or subareas do not need to coincide with the groups and subareas defined by common inlet arrangements.

Common microchannels make it possible to construe microfluidic devices in which the microchannel structures form networks. See for instance US 6,479,299 (Caliper).

Each microchannel structure has at least one inlet opening for liquids and at least one outlet opening for excess of air (vents) and possibly also for liquids.

The microfluidic device of the innovative collection may also comprise microchannel structures that have no reaction microcavity for retaining a solid phase material according to the invention.

- 5 Different principles may be utilized for transporting the liquid within the microfluidic device/microchannel structures between two or more of the functional parts described above. Inertia force may be used, for instance by spinning the disc as discussed in the subsequent paragraph. Other forces are capillary forces, electrokinetic forces, non-electrokinetic forces such as capillary forces, hydrostatic pressure *etc.*

10

The microfluidic device typically is in the form of a disc. The preferred formats have an axis of symmetry ( $C_n$ ) that is perpendicular to the disc plane, where  $n$  is an integer  $\geq 2$ , 3, 4 or 5, preferably  $\infty$  ( $C_\infty$ ). In other words the disc may be rectangular, such as squaric, and other polygonal forms but is preferably circular. Once the proper disc format has been

- 15 selected centrifugal force may be used for driving liquid flow. Spinning the device around a spin axis that typically is perpendicular or parallel to the disc plane may create the necessary centrifugal force. In the most obvious variants at the priority date, the spin axis coincides with the above-mentioned axis of symmetry.

- 20 For preferred centrifugal-based variants, each microchannel structure comprises one upstream section that is at a shorter radial distance than a downstream section relative to the spin axis. A reaction microcavity that contains affinity ligand  $L$  is at a radial position intermediary to these two kinds of sections and typically oriented with the flow direction radially outwards from the spin axis.

25

The preferred devices are typically disc-shaped with sizes and/or forms similar to the conventional CD-format, e.g. sizes that are in the interval from 10% up to 300 % of the conventional CD-radii. The upper and/or lower sides of the disc may or may not be planar.

- 30 Microchannels/microcavities of a microfluidic devices may be manufactured from an essentially planar substrate surface that exhibits the channels/cavities in uncovered form that in a subsequent step are covered by another essentially planar substrate (lid). See WO

9116966 (Pharmacia Biotech AB) and WO 0154810 (Gyros AB). Both substrates are preferably fabricated from plastic material, e.g. plastic polymeric material.

The fouling activity and hydrophilicity of inner surfaces should be balanced in relation to  
5 the application. See for instance WO 0147637 (Gyros AB).

The terms "wetable" (hydrophilic) and "non-wetable" (hydrophobic) contemplate that a surface has a water contact angle  $\leq 90^\circ$  or  $\geq 90^\circ$ , respectively. In order to facilitate efficient transport of a liquid between different functional parts, inner surfaces of the individual  
10 parts should primarily be wettable, preferably with a contact angle  $\leq 60^\circ$  such as  $\leq 50^\circ$  or  $\leq 40^\circ$  or  $\leq 30^\circ$  or  $\leq 20^\circ$ . These wettability values apply for at least one, two, three or four of the inner side-walls of a microconduit. In case one or more of the side-walls have a higher water contact angle this can be compensated for by a lower water contact angle for the remaining side-wall(s). The wettability, in particular in inlet arrangements should be  
15 adapted such that an aqueous liquid will be able to fill up an intended microcavity by capillarity (self suction) once the liquid has started to enter the cavity. A hydrophilic inner surface in a microchannel structure may comprise one or more local hydrophobic surface breaks in a hydrophilic inner side-wall, for instance for introducing a passive valve, an anti-wicking means, a vent solely function as a vent to ambient atmosphere etc. See for  
20 instance WO 9958245 (Gyros AB) and WO 0274438 (Gyros AB).

Contact angles refer to values at the temperature of use, typically  $+25^\circ\text{C}$ , are static and measured by the method given in WO 0056808 (Gyros AB) and WO 0147637 (Gyros AB).

## 25 Packages of the various components of the collections.

The microfluidic devices are typically delivered to the customer with the solid phase preloaded to the different reaction microcavities. The solid phase material in each reaction microcavity may be in a wet state but is preferably in a dry state (which includes also a dehydrated state) for future reconstitution by the customer. Solid phase material in dry  
30 state typically includes one or more agents that stabilize the solid phase material as such and/or stabilize the affinity ligand L from damages during a possible freezing, drying, storage and reconstitution (bed-preserving agents, cryostabilisators, lyostabilisators,

stabilisators). See for instance our copending patent application filed in parallel with this application ("Pre-loaded microscale devices") and Arakawa et al (Advanced Drug Delivery Reviews 46 (2001) 307-326). An important variant of bed-preserving agents is microcavity adherence agents. This kind of agents comprises a compound or a mixture of compounds  
5 that promote retaining of a solid phase material in a microcavity by increasing the adherence between the material and inner surfaces of the microcavity and/or between particles if the material is in particle form. A microcavity adherence agent typically comprises carbohydrate or polymer structure.

10 Other reagent components of the collection may also be provided in solution or in a dry state of the same kinds as discussed in the preceding paragraph. This applies for instance to the conjugate, the binder B in unconjugated form, other affinity reactants (if present) etc.

The various sets of the innovative collection is typically included sales material that is  
15 common for the sets, i.e. sales material that present two or more of the sets in parallel.

#### SECOND ASPECT: THE USE OF THE COLLECTION.

This aspect comprises a method for carrying out a plurality of the affinity reactions, for instance in parallel. Each of the reactions is between the immobilized form of an affinity  
20 counterpart AC's and a solute S' where AC's and S' are affinity counterparts to each other.

The steps of the method are:

- (i) selecting the appropriate set in the innovative collection, and
- (ii) selecting a microfluidic device comprising microchannel structures of the selected set,
- 25 (iii) providing a conjugate B-AC's and S'
- (iv) sensitizing the solid phase in the reaction microcavities of at least a subgroup of the microchannel structures of the set on the selected microfluidic device with the conjugate provided in step (iii),
- (v) performing the affinity reaction.

30

The various embodiments discussed in the context of the first aspect of the invention are applicable also to the second aspect.

**Static versus flow conditions in the porous bed during use.**

The interaction between the immobilized affinity counterpart AC<sub>s</sub> and the solute S may take place under static or flow conditions, i.e. with or without transport of the solute by a liquid flow passing through the reaction microcavities. We have previously found that more information may be gained under flow conditions (WO 0275312 (Gyros AB), for instance with the flow rate and/or residence time adjusted to give conditions such that the distribution of bound solute in the solid phase will reflect the actual reaction rate or affinity between an immobilized affinity reactant, typically AC<sub>s</sub>, and a solute, typically solute S, with a minimum of perturbation by diffusion (non-diffusion limiting conditions). This also applies to the present invention but does not exclude that for applications where the primary interest is the total amount of bound/captured solute, capturing under flow conditions utilizing either diffusion limiting or non-diffusion limiting conditions can be used. The appropriate flow rate through the porous bed thus depends on a number of factors, e.g. the immobilized reactant and the solute and their sizes, the volume of the reaction microcavity, the porous bed including the solid phase material etc. Typically the flow rate should give a residence time of  $\geq 0.010$  seconds such as  $\geq 0.050$  sec or  $\geq 0.1$  sec with an upper limit that typically is below 2 hours such as below 1 hour. Illustrative flow rates are within 0.01-1000 nl/sec, such as 0.01-100 nl/sec and more typically 0.1 - 10 nl/sec. These flow rate intervals may be useful for bed volumes in the range of 1-200 nl, such as 1-50 nl or 1-25 nl. Residence time refers to the time it takes for a liquid aliquot to be in contact with the solid phase in the reaction microcavity.

**The affinity reaction**

The affinity reaction may be part of an assay protocol or a separation protocol of the kind specified in the introductory part. In the most typical case the affinity reaction is part of an assay for the characterization of one or more reaction variables.

Reaction variables that are to be characterized by the use of affinity reactions are mainly of two kinds 1) variables related to affinity reactants, and 2) reaction conditions. The first category has two main subgroups a) amounts including presence and/or absence, concentration, relative amounts, activity such as binding activity and enzyme activity, etc,

and b) properties of affinity reactants including affinity as such, e.g. affinity constants, specificities etc. The molecular entity for which a reaction variable of type 1 is characterized is called an analyte. See WO 0275312 (Gyros AB).

- 5 The term "analyte" in the context of the present invention refers to an uncharacterized molecular entity that is present in a liquid sample and is to be characterized with respect to amount and/or to chemical or biochemical properties. The term includes analyte-derived entities that emanate from an original analyte in an original sample that has been processed to a sample used in a microfluidic device. This preprocessing may take place outside the
- 10 microfluidic device and/or in separate substructures within the microfluidic device. Preprocessing may include that an original analyte participates in affinity reactions, enzymatic and/or chemical conversion etc. The analyte-derived entity may have a chemical composition that is different from the original analyte, be an affinity complex or an uncomplexed affinity reactant that differ from the original analyte etc. The presence and
- 15 amount of the analyte-derived entity is always related to the occurrence of the original analyte in the original sample. The solute S may be an analyte-derived entity or the original analyte of the original sample.

The second category of reaction variables includes pH, temperature, ionic strength,

20 presence of hydrogen bond breaking agents, detergents, liquid flow, immobilization techniques, solid phases etc for the affinity reaction studied. See WO 0275312 (Gyros AB).

- The assay reaction studied concerns formation or dissociation of an affinity complex. The
- 25 characterization typically involves: a) Determination of an uncharacterized amount of an analyte in a sample, b) Selection of binders (analytes) from a library of potential binder candidates, c) Determination of immobilization techniques and/or solid phases that are optimal for a given affinity pair, D) Determination of suitable reaction conditions related to the liquid in which the reaction is carried out, e) Determination of a ligand and/or a binder
- 30 with respect to their suitability for the dissociation of their affinity complex, and f) Determination of qualitative aspects of complex formation, etc. See WO 02075312 (Gyros AB) for further details.

**Major assay protocols.**

In a typical case an uncharacterized amount of an analyte of a sample is allowed to form an affinity complex comprising at least the analyte and an affinity counterpart to the analyte  
5 (anti-analyte). Depending on the protocol used, additional affinity reactants may be used and possibly also incorporated into the complex. The amount and type of reactants are selected so that the affinity reactions involved will result in an amount of the affinity complex that will reflect the true binding ability and/or the amount of the analyte in the sample. See for instance WO 0205312 (Gyros AB).

10

These assay protocols typically utilize an analytically detectable reactant and/or an immobilized or immobilizable reactant. Either one or both of these reactants are fully or partly incorporated into the complex comprising the analyte. Immobilization is used to  
15 separate the complex comprising the analyte from reactants not incorporated in the complex, for instance to separate a complex comprising an analytically detectable reactant from uncomplexed forms of the same reactant.

There are mainly two kinds of assay protocols that can be applied to the innovative collection of microfluidic devices.

20

**Competitive/inhibition protocols.**

In these protocols the analyte and an analyte analogue are competing with each other for binding to a limiting amount of an anti-analyte. The anti-analyte may be

- 25 (a) immobilized or immobilizable if the analyte analogue is soluble and analytically detectable, and  
(b) analytically detectable if the analyte analogue is immobilized or immobilizable.

Depending on the exact protocol used the immobilized or immobilizable anti-analyte may correspond to affinity counterpart AC<sub>s</sub> and the analyte and/or the analyte analogue to  
30 solute S in variant (a). For variant (b), the immobilized or immobilizable analyte analogue may correspond to affinity counterpart AC<sub>s</sub> and the analytically detectable anti-analyte analogue to solute S.



Among competitive variants, alternative (b) is of great interest for the invention at the priority date. This variant includes that the analyte and the anti-analyte are preincubated before reaching the reaction microcavity, for instance outside the microfluidic device or in  
5 a separate mixing microcavity upstream the reaction microcavity. The mixture is transported through the reaction microcavity where the free (=uncomplexed) anti-analyte forms an affinity complex with an immobilized analyte analogue. This complex is subsequently measured. The uncomplexed anti-analyte may be regarded as solute S and the immobilized analyte analogue then corresponds to affinity counterpart AC<sub>S</sub>.

10

Competitive variants include displacement assays in which an immobilized or immobilizable affinity complex comprising an analyte analogue and an anti-analyte is incubated with an analyte (= solute).

### 15 Non-competitive protocols

These protocols typically utilize non-limiting amounts of one or more affinity counterparts to the analyte.

The most important non-competitive protocols are of the sandwich-type and comprises  
20 formation of immobilized or immobilizable complexes in which an analyte is sandwiched between two anti-analytes (that are equal or analogues to each other). One of the anti-analytes is analytically detectable and the other immobilized or immobilizable and possibly also analytically detectable. Depending on the exact protocol used the immobilized or immobilizable anti-analyte may correspond to affinity counterpart AC<sub>S</sub>. The analyte, or the  
25 complex between the analyte and the other anti-analyte, or the uncomplexed form of the other anti-analyte may then correspond to solute S.

Another non-competitive variant utilizes only one anti-analyte, which is in immobilized or immobilizable form. In this case complex formation leads to an immobilized complex, or a  
30 soluble complex that subsequently is immobilized. The immobilized complex as such is then measured. In a typical variant the anti-analyte corresponds to affinity counterpart AC<sub>S</sub> and the analyte to solute S.

**Analytically detectable reactants.**

- By the term "analytically detectable" is contemplated that an affinity reactant can be analytically discriminated from other affinity reactants participating in the formation of the complex to be measured. Detectability may derive from an inherent property of the reactant, for instance an inherent biological function such as the enzyme activity of an enzyme or Fc-receptor binding activity of various Ig-classes and subclasses, or a separately introduced functionality, e.g. labeling with an analytically detectable tag or label, such as biotin (= affinity label), enzyme, chromogen, fluorogen, fluorophor, chemiluminescent group, radioactive group etc.

Formed complex as such may also be detectable, for instance by changing the optic properties of a solution, of a surface etc.

- A detectable label may be combined with a second label selected such that the labels together give the appropriate signal when the complex is formed or dissociated. This variant may be illustrated with so-called scintillation proximity assays (SPA) and by using pairs of interacting fluorophores.

**EXPERIMENTAL PART****FIGURES**

Figure 1 gives a subgroup of microchannel structures of the microfluidic device utilized in the experimental part.

Figure 2 shows the dynamic capacity for different kind of solid phase material in particulate form: Superose 20 mg/ml IPA (1), Superose 2mg/ml (2), Sperdex P IPA (3), Polystyrene-PheDex (4), Sepharose HP IPA (5). Fluorescence intensity is given in radial direction through the bed (length of the bed) with the peak typically at the entrance. Affinity ligand L is Streptavidin. The effect is measured in the fluorescence myoglobin immunoassay given below with a concentration of myoglobin of 500nM with streptavidin as the immobilized affinity ligand L.

Figures 3 shows the result with different binding capacity of a solid phase material of different binding capacities: 5.5 mg streptavidin/ml porous bed (1), 0.58 mg streptavidin/ml porous bed (2), and 0.08 mg streptavidin/ml porous bed (3). Measurement as for figure 2.

5

The microfluidic device used for the experiments was circular and of the same dimension as a conventional CD (compact disc). The microfluidic device will henceforth be called CD in the experimental part. The CD contained 14 groups of 8 microchannel structures arranged in an annular zone around the center (spin axis) of the disc with a common waste  
10 channel for each group close to the periphery. A group of 8 microchannel structures is shown in figure 1 and is similar to and function in the same manner as the subgroup illustrated in figures 1-2 of (WO 0275312, Gyros AB) and the corresponding figures in PCT/SE02/01678 (Gyros AB) and PCT/SE02/01701 (Gyros AB). The dimensions are essentially of the same size as in these earlier patent applications.

15

Each subset (100) comprises 8 microchannel structures (101a-h), one common inlet arrangement (102) and eight separate inlet arrangements (103a-h), and eight reaction microcavities (104a-h). The common inlet arrangement comprises a) two inlet ports (105a-b) that also will function as outlet port for excess liquid, and b) one volume-metering unit  
20 (106a-h) for each microchannel structure (101a-h). The volume-metering units (106a-h) will function as a distribution manifold for the downstream parts of the microchannel structures. Each of the separate inlet arrangements (103a-h) is part of only one microchannel structure and comprises an inlet port (107a-h) and a volume-metering unit (108a-h). Between each volume-metering unit (106a-h, 108a-h) and their downstream  
25 parts, respectively, there is a valve function (109a-h, 110a-h), preferably passive. A reaction macrocavity (104a-h) of a microchannel structure (101a-h) is located downstream both the common inlet arrangement (102) and the separate inlet arrangement (103a-h) of a microchannel structure (101a-h). At the outlet end (111a-h) of each reaction microcavity, the depth is lowered from 100 µm to 10 µm in two steps to prevent particles from escaping  
30 the reaction microcavity. At the periphery there is a common waste channel (112). Vents (113a-i) together with the valves (109a-h) define the volume of the liquid aliquots to be distributed downstream from each the volume-metering unit (106a-h).

By applying the appropriate volume of aqueous liquid to the inlet port of an inlet arrangement, capillarity will fill the volume-metering unit(s) connected to the inlet port with liquid. By spinning the disc around its center, liquid can be forced to pass the valve  
5 between a volume-metering unit and downstream parts.

## EXPERIMENTALS

### Instrumentation

The immunoassay was performed in an automated system. The system (Gyrolab  
10 Workstation, prototype 2 instrument equipped with a Laser Induced Fluorescence (LIF) module, Gyros AB, Uppsala, Sweden) was equipped with a CD-spinner, holder for microtiter plates (MTP) and a robotic arm with a holder for 10 capillaries connected to 5 syringe pumps, 2 and 2. Two of the capillaries transferred all the reagents and buffers from a MTP to either of the two common inlet ports (105a-b) in the CD. The other eight  
15 capillaries transferred individual samples from a MTP to the separate individual inlet ports (107a-h) in the CD.

The Gyrolab Workstation is a fully automated robotic system controlled by application-specific software. An application specific method within the software controls the spinning  
20 of the CD at the precisely controlled speeds and thereby controls the movement of liquids through the microstructures as the application proceeds. Special software was included in order to reduce background noise.

See also WO 02075312 (Gyros AB), PCT/SE02/01678 and US 10/062,258 (Gyros AB),  
25 and PCT/SE02/02455 and 10/331,399 (Gyros AB) and also [www.gyros.com](http://www.gyros.com).

### Preparation of porous beds.

For example solid polystyrene particles (15 mm, Dynal Biotech, Oslo, Norway) were selected for the solid phase. The beads were modified by passive adsorption of phenyl-  
30 dextran to create a hydrophilic surface and were subsequently covalently coupled with streptavidin (Immunopure Streptavidin, Pierce, Perbio Science UK Limited, Cheshire, United Kingdom) using CDAP chemistry (Kohn & Wilchek, Biochem. Biophys. Res.

Commun. 107 (1982), 878-884). Solid phase material in the form of porous particles (beads) were Superose, Superdex Peptide (Superdex P) and Sepharose HP (Amersham Biosciences, Uppsala, Sweden) have also been covalently coupled with streptavidin using CDAP chemistry (without phenyl-dextran coating). The polystyrene particles are solid and non-swellable, and Superose, Superdex Peptide and Sepharose HP are porous in relation to many affinity reactants and swellable in the liquids used.

After coupling with the streptavidin, a suspension of the particles in potassium phosphate buffer (10mM) was distributed in the common distribution channel (102) via inlet port (105a-b) and moved through the structure by centrifugal force. The centrifugal force combined with the vents (109a-h, 113a-i) divide the suspension in the common inlet arrangement (102) in equal portions, each of which forms a bed of packed particles (column) in each reaction microcavity (104a-h) against the dual depth (111a-h). The approximate volume of the column was 15 nl.

15

#### Immunoassay

The catching antibody in our myoglobin assay, the monoclonal antimyoglobin 8E11.1 (LabAS, Tartu, Estonia) was biotinylated using Sulfo-NHS-LC-biotin (Pierce, prod # 21335, Perbio Science UK Limited, Cheshire, United Kingdom). The protein concentration of the monoclonal antimyoglobin 8E11.1 was 1-10 mg/ml and it was incubated in room temperature for 1 h with 3× molar excess of the biotinylation reagent in 15 mM PBS with 0,15 M NaCl before it was gel filtrated through either a NAP-5 column (Amersham Biosciences, Uppsala, Sweden) or a Protein Desalting Spin Column (Pierce, # 89849-P, Perbio Science UK Limited, Cheshire, United Kingdom).

25

To load the streptavidin immobilized particles with the biotinylated antibody, a solution at a 0,2-2 mg/ml concentration (depending of how much streptavidin it is in the packed column) of antibody was distributed in the common distribution channel via inlet port (105a-b) and moved through the structure by centrifugal force. The flow rate through the columns was controlled by the spin velocity (spin flow 1). After the capturing antibody was attached to the columns they were washed once by addition of PBS (with 0,01%

30

Tween 20) to the common distribution channel (inlet ports 105a or b) followed by a spin step.

To demonstrate the myoglobin assay in Gyrolab Workstation a 6-point standard curve was created. The myoglobin samples (diluted in PBS with 1% BSA) of 500 nM each were distributed to the individual inlet ports (107a-h). The sample volume 200 nl was defined into the volume-metering unit (108a-h), during the first two steps in the spin flow method. To reach favourable kinetic condition under the capturing step (for myoglobin to bind to 8E11.1) the flow rate of the sample should not exceed 1 nl/sec. The sample flow rate was controlled by spin flow 2. After sample capturing the columns was washed twice by addition PBS (with 0,01% Tween 20) to the common distribution channel (inlet port 105a or b) followed by a spin step. Detecting antibodies (monoclonal antimyoglobin 2F9.1 (LabAs, Tartu, Estonia)) in excess were applied next via the common distribution channel (inlet port 105a or b) and a similar slow flow rate (spin flow 3) was used. The detecting antibodies were labeled with a fluorophore Alexa 633 (Molecular Probes, Eugene, USA). Excess of labeled antibody was washed away by 4 additions of PBS (with 0,01% Tween 20) to the common distribution channel (inlet port 105a or b) followed by a spin step.

The complete assay was analyzed in the Laser Induced Fluorescence (LIF) detector module. See more WO 02075312 (Gyros AB), PCT/SE02/01678 and US 10/062,258 (Gyros AB), and PCT/SE02/02455 and 10/331,399 (Gyros AB).

An overview of the run method performed in the system is presented in Table 1.

## 25 Results

Figure 2b illustrates that solid phase comprising different base matrices could be use. The only base matrix that deviates for the concentrations used is PS-PheDex.

Figure 3 illustrates that solid phase material comprising the same base matrix but with different concentration of immobilized affinity ligand L (streptavidin in this case) could be used. If due care is taking to background and noise the integrated fluorescence for labeled antibody will be the same for all three concentrations of streptavidin.

Table1

METHOD	SPIN PROFILE
Rewetting of bead columns	
Spin 1	2500 rpm 5s, 6000 rpm 10s
Wash of beads	
Spin 2	1200 rpm 2s, 2500 rpm 0,5s, 4000 rpm 10s, 6500 rpm 16s
Transfer of biotinylated antibody	
Spin flow 1	1200 rpm 2s, 2500 rpm 0,5s, from 1200- 1500 rpm 45s, 2000 rpm 35s, 3000 rpm 30s, 4000 rpm 10s, 5000 rpm 5s, 6000 rpm 10s
Wash of beads and CD-structure 1	
Spin 3	1200 rpm 2s, 2500 rpm 1s, 4000 rpm 15s, 6000 rpm 18s
Transfer of myoglobin samples	
Spin flow 2	1000 rpm 5s, 2500 rpm 0,5s, from 1200- 1500 rpm 90s, 2000 rpm 70s, 3000 rpm 60s, 4000 rpm 20s, 5000 rpm 10s
Myoglobin wash 1	
Spin 4	1200 rpm 2s, 2500 rpm 0,5s, 4000 rpm 10s, 6000 rpm 16s
Myoglobin wash 2	
Spin 5	1200 rpm 2s, 2500 rpm 0,5s, 4000 rpm 10s, 6000 rpm 16s
Transfer of conjugate	
Spin flow 3	1200 rpm 2s, 2500 rpm 0,5s, from 1200- 1500 rpm 90s, 2000 rpm 70s, 3000 rpm 60s, 4000 rpm 20s, 5000 rpm 10s
Conjugate wash 1	
Spin 6	1200 rpm 2s, 2500 rpm 0,5s, 4000 rpm 10s, 6000 rpm 16s

Conjugate wash 2	
Spin 7	1200 rpm 2s, 2500 rpm 0,5s, 4000 rpm 10s, 6000 rpm 16s
Conjugate wash 3	
Spin 8	1200 rpm 2s, 2500 rpm 0,5s, 4000 rpm 10s, 6000 rpm 16s
Conjugate wash 4	
Spin 9	1200 rpm 2s, 2500 rpm 0,5s, 4000 rpm 10s, 6000 rpm 16s
Detection	

#### Drying and reconstitution

Certain innovative aspects of the invention are defined in more detail in the appending

5. claims. Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined by the appended claims. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition

10 of matter, means, methods and steps described in the specification. As one of ordinary skill in the art will readily appreciate from the disclosure of the present invention, processes, machines, manufacture, compositions of matter, means, methods, or steps, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein

15 may be utilized according to the present invention. Accordingly, the appended claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, means, methods, or steps.



**CLAIMS**

1. A collection of one or more microfluidic devices which together carry a plurality of microchannel structures each of which comprises a reaction microcavity in which there is a solid phase with an immobilized affinity ligand L,  
5 characterized in that
- (i) the plurality is divided into sets of microchannel structures, and
  - (ii) the affinity ligand L is directed to the same counterpart (binder, B) independent of set, and
  - 10 (iii) the sets differ in
    - a) the capacity per reaction microcavity and/or the capacity/unit volume of the solid phase in a reaction microcavity for binder B, and/or
    - b) the base matrix of the solid phasediffer between the sets but is equal within each set.
- 15
2. The collection according to claim 1, characterized in that the difference is with a factor  $\geq 1.2$  for at least 1 of the sets of the collection compared to the binding capacity for the set having the lowest binding capacity.
- 20
3. The collection according to any of claims 1-2, characterized in that at least one of said devices comprisesn
- a) at least two of said sets of microchannel structures, and/or
  - b) only one set of microchannel structures, with the proviso that the collection comprises two or more devices which are different with respect to the kind of sets
- 25 they carry.
4. The collection according to any of claims 1-3 being intended for separately performing one or more affinity protocols that differ with respect to the reactants involved and/or the order of addition of the reactants and/or the concentration range in which the reactants are used, each of said different protocols utilizing an affinity reaction
- 30 between
- (i) a solute S, and
  - (ii) a conjugate comprising

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(a) a binder B, and

(b) an affinity counterpart  $AC_S$  to the solute S,

characterized in that the affinity constant  $K_{L-B}$  for formation of the complex L-B between the affinity ligand L and the binder B, i.e.  $K_{L-B} = [L][B]/[L-B]$ , is at most  $10^3$  times, such as at most  $10^2$  times, the corresponding affinity constant for streptavidin and biotin.

5 5. The collection according to claim 4, characterized in that L is amongst biotin-binding compounds and streptavidin-binding compounds, respectively, or vice versa.

10

6. The collection of any of claims 4-5, characterized in that L has two or more binding sites for B.

7. The collection according to any of claims 1-6, characterized in

15 (a) that each set on a device is grouped into one or more groups of fluidly equivalent microchannel structures, and

(b) that each group is located to a particular subarea of the device.

8. The collection according to any of claims 1-7, characterized in that said reaction microcavity in at least one, preferably all, of said microchannel structures in the upstream direction is connected to a volume-metering unit.

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9. The collection according to claim 7, characterized in that said volume-metering unit is part of an inlet arrangement for liquid.

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10. The collection according to each of claims 6-8, characterized in that said volume-metering units within at least one of said group(s) are part of a distribution manifold for distributing liquid to the reaction microcavities of the group, with the proviso that each of said at least one group comprises two or more microchannel structures.

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11. The collection according to each of claims 7-10, characterized in that the inner wall of each of said volume-metering units have a sufficient hydrophilicity for being filled

by capillarity once an aqueous liquid have entered the unit, and b) a valve at its outlet, for instance a passive valve.

- 5 12. The collection according to any of claims 4-11, characterized in that at least one of the solute S and its affinity counterpart ACs, and/or at least one the binder B and the ligand L comprise a structure selected amongst peptide structure including poly/oligo-peptide and protein structure, carbohydrate structure, lipid structure including steroid structure, nucleotide structure including nucleic acid structure, and polymeric structure.
- 10 13. The collection according to any of claims 1-12, characterized in that said solid phase is in a dry state, preferably comprising in addition to the solid phase one or more bed-preserving agents.
- 15 14. The collection according to claim 13, characterized in that at least one of said one or more bed-preserving agents is a microcavity adherence agent.

**ABSTRACT**

A collection of one or more microfluidic devices which together carry a plurality of microchannel structures each of which comprises a reaction microcavity in which there is a solid phase with an immobilized affinity ligand L,  
5 characterized in that

- (i) the plurality is divided into sets of microchannel structures, and
- (ii) the affinity ligand L is directed to the same counterpart (binder, B) independent of set, and
- 10 (iii) the sets differ in
  - a) the capacity per reaction microcavity and/or the capacity/unit volume of the solid phase in a reaction microcavity for binder B, and/or
  - b) the base matrix of the solid phasediffer between the sets but is equal within each set.

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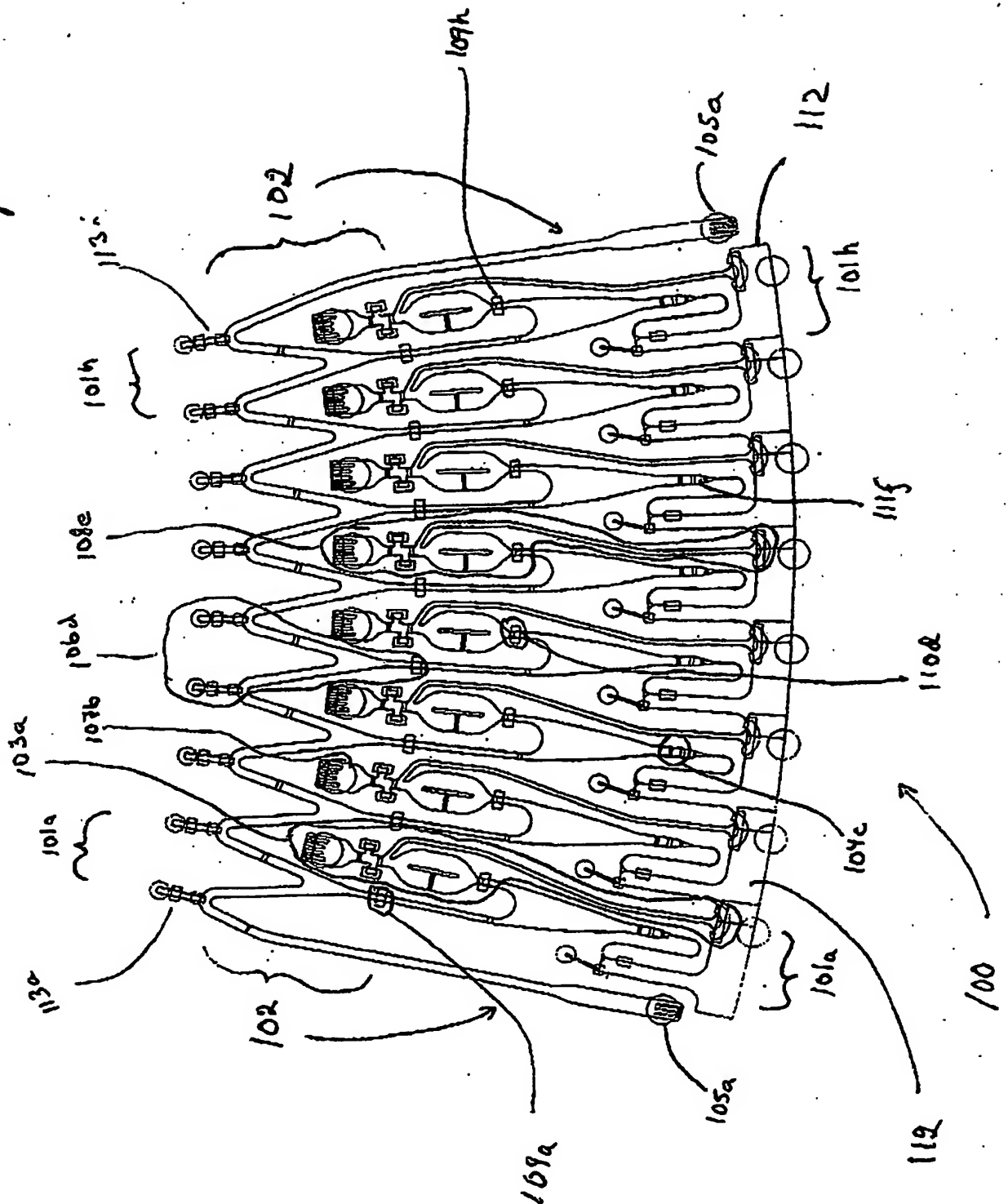
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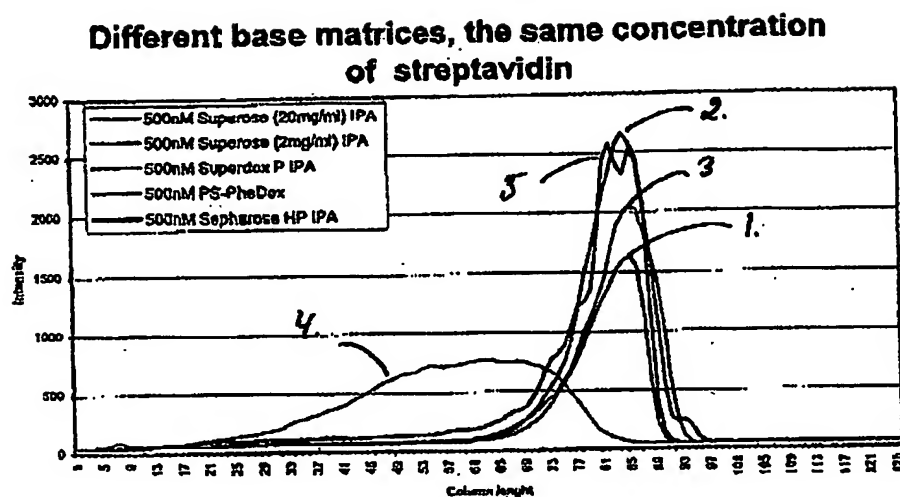
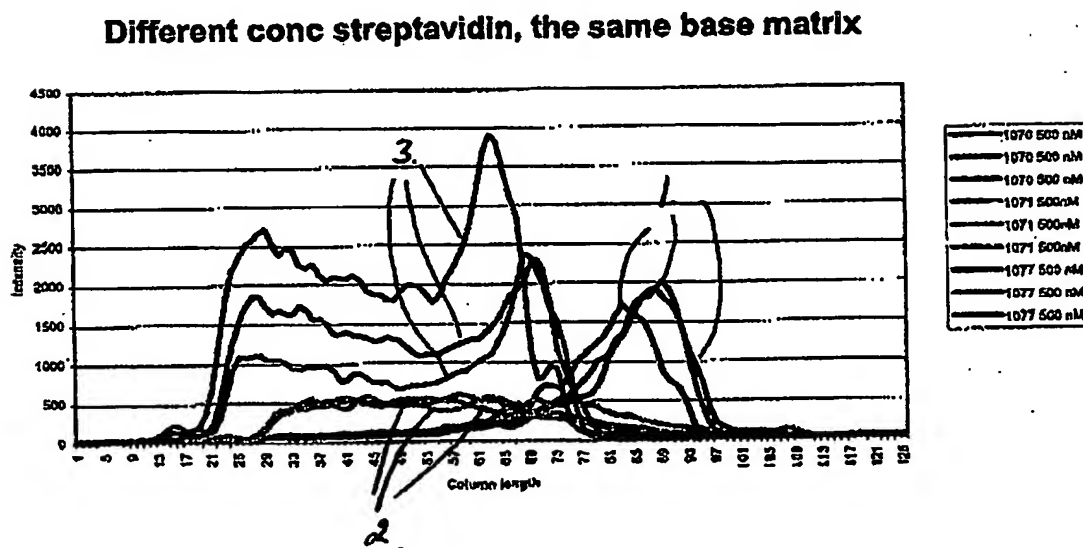
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Fig. 1.



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**Figure 2****Figure 3**

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